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Effect of hyperthermia on histamine blood level and convulsive behavior in infant rats

Peyman Gholipoor [a], Ehsan Saboory [b,*], Shiva Roshan-Milani [b], Javid Fereidoni [c]

[a] Department of Physiology, Faculty of Medicine, Urmia University of Medical Sciences, Urmia, Iran
[b] Neurophysiology Research Center, Urmia University of Medical Sciences, Urmia, Iran
[c] Department of English Language, Faculty of Medicine, Urmia University of Medical Sciences, Urmia, Iran

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ABSTRACT

Febrile seizures (FS), which have been extensively studied using animal models, are the most common type of convulsive events in children, but the cellular mechanisms causing FS are still unclear. Histamine has been suggested to participate in seizure control. This study investigated the effect of hyperthermia (HT) on histamine blood level (HBL) and convulsive behavior in prepubertal rats. Forty Wistar rat pups were assigned to 5 groups ($n = 8$), namely, control, HT, cromolyn, chlorpheniramine, and ranitidine. Two groups of adult rats were also used as control and HT adults. The control rats were placed in a hyperthermic chamber, and a room temperature current of air was blown on them. In all other groups, the rats were placed in the chamber for 30 min, and a current of warm air was applied to them. In the pretreatment groups, the rats received an injection of 68-mg/kg cromolyn sodium, 4-mg/kg chlorpheniramine, or 80-mg/kg ranitidine intraperitoneally 30 min prior to HT. Body temperature and convulsive behaviors were recorded. Then, the rats were anesthetized with ether, and their blood sample was obtained through direct heart puncture. Hyperthermia initiated convulsive behaviors in infant rats but not in the adult ones. Pretreatment with chlorpheniramine significantly potentiated convulsive behaviors ($p = 0.017$). Hyperthermia led to a significant decrease in the HBL of both infant ($p < 0.001$) and adult ($p = 0.003$) rats. Pretreatments led to more decrease in the HBL of infant rats ($p < 0.001$). It was concluded that HT could lead to a decrease in HBL, which in turn increases the seizure susceptibility of animals. Histamine may have a pivotal role in hyperthermia-induced seizures.

1. Introduction

Febrile seizures are very frequent in the early life stages of humans [1]. They are classified within special syndromes and are defined as seizures during childhood, generally occurring between 3 months and 5 years of age. Also, they are usually associated with hyperthermia but without any evidence of intracranial infection or history of previous seizures [2]. The cellular and neurochemical mechanisms causing FS are still unclear. Among other mechanisms, central nervous system (CNS) histamine has been suggested to participate in seizure control and thermoregulation [3]. Histamine is synthesized in two distinct pools in the adult rat brain: neurons in the posterior hypothalamic tuberomammillary nucleus [4] and mast cells located primarily in perivascular areas [5]. Histamine regulates a variety of physiological functions involving energy metabolism, hormone regulation, mastication, thermoregulation, learning and memory, arousal state, and immunity [5]. It has also been reported to play an important role in regulating the anticonvulsant mechanism in different animal models of epileptic seizures. Increased histamine levels elevate seizure threshold and reduce the severity and duration of seizures [6], whereas decreased histamine levels have the opposite effect [7]. Out of the four histamine receptors, the histamine 1 (H_1) receptor is suggested to be of importance in decreasing seizure activity. First generation H_1 receptor antagonists, such as ketotifen and chlorpheniramine, elicit epileptiform activity [8]. In addition to these experimental studies, antihistamines are reported to be closely associated with seizure activities not only in children with epilepsy but also in healthy children [9]. An in vivo positron emission tomography imaging study for histamine H_1 receptors has demonstrated increased histamine H_1 receptor binding in the cortex, which suggests a defensive mechanism with an anticonvulsant system to prevent the spread of electrical activity from epileptic foci [10]. In the brain, mast cells are predominantly located perivascularly, especially in the thalamus and hypothalamus [11]. Degranulation of a mast cell, a highly specialized secretory cell and the main repository for histamine in the body [12], contributes to elevated brain histamine levels [13]. It has been demonstrated that thalamic mast cells contribute up to 90% of histamine in the thalamus and

Abbreviations: FS, febrile seizures; HT, hyperthermia; HBL, histamine blood level; CNS, central nervous system; HS, hyperthermia-induced seizure; IP, intraperitoneally; TC, tonic–clonic; BBB, blood–brain barrier; CSF, cerebrospinal fluid; IL.1β, interleukin-1b.

* Corresponding author. Department of Physiology, Faculty of Medicine, Urmia University of Medical Sciences, Urmia, Iran. Tel.: 04412770698; fax: 04412780801.
E-mail addresses: gholipoorpeyman@yahoo.com (P. Gholipoor), saboory@umsu.ac.ir, saboory@yahoo.com (E. Saboory), shivamialni@umsu.ac.ir (S. Roshan-Milani), jfereidoni@yahoo.com (J. Fereidoni).

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up to 50% of the whole brain histamine levels [14]. It has also been reported that warm or cold temperatures lead to mast cell degranulation without the presence of IgE, so-called direct mast cell degranulation [15,16]. The aim of this study was to clarify whether HT can change histamine blood level (HBL) or not. If so, does pretreatment with antihistamines and cromolyn sodium (as a mast cell stabilizer) alter HBL and the intensity of hyperthermia-induced seizure (HS)? By considering the two points that, firstly, histamine has an inhibitory effect on seizure and, secondly, mast cells are an important source of histamine which can be degranulated by high temperature resulting in the release of histamine, it was hypothesized that inhibiting mast cell degranulation and/or blocking histamine effect may affect the presence and/or intensity of HS. Therefore, this study was designed to investigate the effect of HT on convulsive behavior and HBL in infant rats.

2. Materials and methods

2.1. Ethical approval

All the experimental protocols and procedures were followed according to the 1975 Declaration of Helsinki guidelines, as reflected in the guidelines of the Medical Ethics Committee, Ministry of Health, Iran. In addition, the Regional Medical Ethics Committee in West Azerbaijan province, I.R. Iran, approved this study.

2.2. Subjects

Ten pregnant Wistar rats were kept in standard animal house conditions at 22 ± 2 °C with 12-h light–dark cycles and were given unrestricted access to food and water. The day of parturition was considered as day 0. The offspring were mixed at birth, and then, 8 animals (4 males and 4 females) were assigned to each dam. Nineteen- and twenty-day-old male and female rats were used because it has been reported that there is no difference in hyperthermia-induced effects between the two sexes [17]. The 19- and 20-day-old rats were subjected to hyperthermic seizure because these ages are equivalent to the time in which human infants are susceptible to febrile convulsions. It has been reported that 5- to 7-day-old rats are equivalent to full-term newborn infants. The brain of 15-day-old rats is equivalent to the brain of human infants a few months to 1 year of age, and the brain of 28- to 30-day-old rats is equivalent to the brain of 2-year old children [18,19]. Morimoto et al. have pointed out that rats younger than 10 days old are too immature for the study of febrile seizures, and since some parameters of the neurotransmitter system still continue to develop and only reach the same level as that of an adult at the age of 1 month, the brain of a rat younger than 1 month is appropriate for the investigation of febrile seizures in infants [20,21].

2.3. Drugs

The applied drugs were chlorpheniramine and ranitidine purchased from Darou Pakhsh Company (Tehran, Iran) and cromolyn sodium from Sigma-Aldrich, Germany. Cromolyn sodium was dissolved in saline, and it was freshly prepared for daily use. Other drugs were in liquid form and were used in their primary forms, except in dilution with saline for dose adjustment.

2.4. Grouping and intervention

The pups were assigned to five different groups as follows: control, hyperthermia (HT), cromolyn sodium, chlorpheniramine, and ranitidine. Two groups of adult rats were also used in this study as control and HT adults. The control rats were placed in a hyperthermic chamber for 30 min, and a current of room temperature air was blown on them. In all other groups, the rats were placed in the chamber for 30 min, and a current of warm air was blown on them. Meanwhile, in the pretreatment groups, the rats received an injection of 68-mg/kg cromolyn sodium, 4-mg/kg chlorpheniramine, or 80-mg/kg ranitidine intraperitoneally (IP) 30 min prior to HT. All the pups were kept with their dam until 35 min prior to the experiment. Then, the infant rats were weighed and were injected with 10-ml/kg saline IP before placing them in the chamber to prevent dehydration. The core body temperature of the rats was measured by means of a lubricated rectal probe before and during HT. The rectal temperature of each rat was measured at baseline with a lubricated temperature probe inserted into the rectum and connected to a temperature amplifier (Campden, UK). Hyperthermic seizures were induced using the model described previously by Baram et al. [22]. Briefly, 19- and 20-day-old rats were placed one by one on the floor of the hyperthermic chamber, which was 45 cm in height and 30 cm in diameter. A warm air current was blown from the roof of the chamber (at a distance of approximately 40 cm above the rats). The container temperature was monitored near the chamber wall at a height of approximately 2 cm. Air temperature was maintained (±1 °C) by feedback electronics, which automatically adjusted heating levels several times per second in response to the air temperature. When a lower air temperature was required to prevent animals from overheating, the system adapted itself within seconds. Chamber temperature was maintained at 25 °C for control rats and at 48 °C for HT rats. The rectal temperature of the animals was recorded every 2 min. Hyperthermia (40–41 °C) was typically reached within 5 min. When the core temperature of the HT rats reached 43 °C, the air temperature was adjusted to maintain the rats' core body temperature between 42 and 43 °C. Hyperthermia was maintained for 30 min. Then, the rats were partly submerged in water at room temperature to quickly normalize their core body temperature. The controls were treated as HT pups, except that the temperature of the chamber was kept at 25 °C. The control pups were from the same litters as those exposed to HT. After cooling the rats with water (25 °C, 5 min), all the rats were anesthetized with ether, and their blood sample was obtained through direct heart puncture. The samples were immediately transferred to 1.5-ml EDTA-coated microcentrifuge tubes and were kept on ice; later, they were centrifuged for 15 min at 9000 rpm at 4 °C. The plasma was transferred to a clean 1.5-ml microcentrifuge tube and was stored frozen at −70 °C until histamine levels were determined. Histamine level was measured using the ELIZA commercial kit (IBL, Hamburg, Germany), and the values were expressed in nanogram per milliliter.

2.5. Behavioral analysis

To analyze HS susceptibility in rats, only behavioral analysis was performed (see also Table 1). The total time of the behavioral analysis was about 30 min. Core body temperature was quickly raised as described before. Animal behavior was recorded by two cameras, one from the top and another from the side. The observer recorded latencies using stage 2, 3, and 4 behavioral seizures (for descriptions of stages, see Table 1). As seizure behavior was absent for a period of 30 min, the

Table 1
Classification of rat behavior during HT.

Stage	Behavior	Description
0	Normal	Normal explorative behavior
1	Hyperactivity	Hyperactive behavior, jumping, and rearing
2	Immobility	Sudden total immobility (duration: 3–10 s)
	Ataxia	Unsteady, jerky gait
3	Circling	Running in tight circles (approximately two circles/s)
	Shaking	Whole-body shaking
	Clonic seizures	Contractions of hindlimbs and forelimbs with reduced consciousness
4	Tonic–clonic convulsions	Continuous tonic–clonic convulsions

length of the latency period was considered to be 30 min. The intensity of seizure was evaluated by the total score of seizure as follows:

$$TSS = SBS + 1/LTCS \times 100 + NTCS + DTCS$$

where TSS stands for total score of seizure, SBS stands for sum of behavioral stages (Table 1), LTCS is latency for tonic–clonic seizure (min), NTCS refers to the number of tonic–clonic seizures, and DTCS indicates duration of tonic–clonic seizures (min).

For example, if a rat presented behaviors from all the stages shown in Table 1, its SBS would be $1 + 2 + 3 + 4 = 10$; if LTCS was 20, NTCS was 7, and DTCS was 3, then its TSS would be calculated as follows: $TSS = 10 + 1 / 20 \times 100 + 7 + 3 = 25$.

It must be mentioned that because of the lack of proper equipment and because we did not perform an electroencephalographic (EEG) recording simultaneously, seizure behavior at its optimum level could not be described in the present experiments.

2.6. Statistical analysis

The results were expressed as mean ± SEM; meanwhile, the data that were normally distributed (values of histamine and some seizure parameters) were analyzed using parametric techniques. One-way analysis of variance (ANOVA) was used to compare multiple groups, and Tukey's test was used for post hoc analysis. The data that were not normally distributed (values of some seizure parameters) were analyzed using Kruskal–Wallis one-way ANOVA. When appropriate, Dunn's test was used for post hoc analyses. All the tests had a critical significance level of $p < 0.05$.

3. Results

The baseline temperature and final temperature of the rats are presented in Table 2. There was no significant difference between experimental groups at either baseline or final temperature. First, tonic–clonic (TC) temperature was measured for all the rats, which was significantly lower in the chlorpheniramine-treated rats than in the other groups ($p < 0.001$). There was no significant difference between the other groups.

3.1. Behavioral screening of HS susceptibility in infant rats

Hyperthermia-induced seizure susceptibility was analyzed using behavioral screening of the rats. The behavioral range in this screen was classified according to Table 1. Latency until immobility (stage 2), circling (stage 3), and tonic–clonic convulsions (stage 4) were recorded by the observer as a measure for HS susceptibility in the groups. All the rats showed sudden immobility, but in some rats, hyperthermia did not elicit a circling behavior. Therefore, in the cromolyn group, 50% of the rats did not show clear circling; instead, they had a bidirectional movement in a way which was considered stage 3. Other groups demonstrated a clear circling movement. Chlorpheniramine (a H_1 blocker), while used as pretreatment, augmented HS, i.e., the latency of seizure onset was significantly shorter in this group compared to the other groups ($p = 0.001$). However, no significant difference was observed in latency to immobility and circling. To clarify the effect of H_2 receptor in HS, ranitidine (a H_2 blocker) was used 30 min before HT. There was no significant difference in seizure parameters between the ranitidine-treated rats and the HT group. However, there was a mild tendency (nonsignificant) toward decreasing the circling latency compared to the HT group. Cromolyn sodium (a mast cell stabilizer) showed a tendency in accelerating seizure development induced by HT, but the effects were not significant. Table 3 summarizes the behavioral diversity of HS susceptibility in the rats, which reflects particular behavioral latencies during HT.

3.2. Seizure intensity

As described in the Materials and methods section, the total score of seizure was calculated and considered as seizure intensity. However, as shown in Fig. 1, pretreatment with chlorpheniramine significantly increased seizure intensity ($p = 0.01$), while ranitidine and cromolyn showed no marked effect on seizure intensity.

3.3. Effect of HT on histamine blood level

To determine the effect of HT and different pretreatments on histamine, the level of histamine in plasma was measured. Hyperthermia significantly decreased HBL. Pretreatment with ranitidine, chlorpheniramine, and cromolyn remarkably decreased HBL (Fig. 2).

3.4. Effect of HT on adult rats (age and seizure)

To evaluate the importance of age on HT, two groups of adult rats were used. The result of behavioral assessment is given in Table 4. None of the adult rats showed tonic–clonic seizure. Twenty-five percent of adult rats presented a circling behavior. The rest (75%) only showed immobility. To determine the effect of HT on HBL in adult rats and to compare it with the values in infant rats, HBL was measured in control and HT adult rats. Histamine blood level was significantly lower in control adults than in control pups. Hyperthermia significantly decreased HBL in adult rats as well (Fig. 3).

4. Discussion

The most important finding of this study was as follows: HT initiated convulsive behaviors in infant rats but not in adult rats; meanwhile, pretreatment with chlorpheniramine potentiated convulsive behaviors. Hyperthermia led to a significant decrease in the HBL of both infant and adult rats, while pretreatments led to further decrease in the HBL of infant rats (considering pretreatment, the adult rats were ignored because of financial limitations). An HS susceptibility screening was used in infant rats, and this screening assay took 30 min per animal. Also, it was highly sensitive because, within this period, the rat pups showed complete HT-induced behavioral repertoire (Tables 2 and 3) starting with immobility, circling and shaking, and tonic–clonic convulsion. Heat-induced immobility is considered the first sign of HS [23].

4.1. Effect of cromolyn on HS

The present data showed that cromolyn had no significant effect on seizure intensity, which may be attributed to the diversity of cromolyn

Table 2
Values of core body temperature in infant rats during HT.

Parameter	Control	Hyperthermia	Chlorpheniramine	Ranitidine	Cromolyn
Baseline temperature	36.37 ± 0.13	36.38 ± 0.15	36.10 ± 0.11	36.30 ± 0.10	36.22 ± 0.10
First TC temperature	–	42.61 ± 0.21	42.30 ± 0.10*	42.66 ± 0.20	42.55 ± 0.13
Final temperature	36.43 ± 0.15	42.95 ± 0.03	42.93 ± 0.04	42.92 ± 0.06	42.97 ± 0.04

Infant rats were exposed to HT for 30 min, and their core body temperature was recorded by a rectal probe.
* $p < 0.001$ with all groups ($df = 3$, $F = 12.4$, ANOVA).

Table 3
Behavioral diversity of HS susceptibility in different groups.

Parameter	Hyperthermia	Chlorpheniramine	Ranitidine	Cromolyn
Immobility latency	8.62 ± 0.46	7.25 ± 0.31	7.87 ± 047	8.25 ± 0.36
Circling latency	16.12 ± 0.25	13.50 ± 0.42	15.37 ± 0.65	15.00 ± 0.33
TC latency	26.37 ± 0.59	22.00 ± 0.46*	25.50 ± 0.46	25.37 ± 0.59

Infant rats were placed in a hyperthermic chamber for 30 min. A warm air current was blown on the rats. In the chlorpheniramine, ranitidine, and cromolyn groups, the rats were pretreated with these drugs 30 min prior to HT.
* $p < 0.001$ with all groups ($df = 3$, $F = 11.92$, ANOVA).

actions in the brain. Cromolyn is a mast cell stabilizer which may not only block histamine release in the mast cell but is also responsible for blood–brain barrier (BBB) permeability; it releases cytokines that can be diffused to other brain areas and increases BBB permeability. Stabilization of mast cell reduces histamine release, and reduction of histamine, in turn, potentiates HS. The result of the current study is consistent with the findings in the literature. As illustrated in Fig. 2, HBL decreased in cromolyn-treated rats. Cromolyn has been shown to have an antiseizure effect in mice. Confirming this result, it has been reported that cromolyn attenuates amisulpride-induced seizurogenic effect [24]. Thus, it can probably be concluded that the antiseizure effect of cromolyn interacted with its proseizure effect (by decreasing histamine release). Therefore, no robust effect of cromolyn on HS was observed.

4.2. Effect of chlorpheniramine on HS

Histamine in the brain is involved in the termination of seizures and plays an endogenous anticonvulsant role in epilepsy. It is supported by the fact that seizures are sometimes induced in childhood epilepsy by treatment with an antihistamine drug [25]. It has been reported that pyrilamine, ketotifen, and D-chlorpheniramine increase duration of tonic–clonic and convulsive coma phase following electroconvulsions in 21- and 30-day-old mice but not in 42-day old animals, while peripherally acting H_1 receptor antagonists have no influence on these parameters [26]. Our finding is in line with the result of these investigators. In the current study, temperature thresholds for seizures and latency for tonic–clonic seizure were decreased, while seizure intensity significantly increased in the chlorpheniramine group compared to the HT group (Tables 2 and 3). These novel findings suggested that the H_1 receptor may play a pivotal role in the regulation of HT-induced seizure susceptibility (and threshold, of course) in immature rat brains. First-generation antihistamines may increase seizure susceptibility by acting directly on ion channels in CNS neurons [27]. According to the clinical experiences described above as well as the present results, it can be suggested that histamine has an inhibitory role in HS via histamine H_1 receptors.

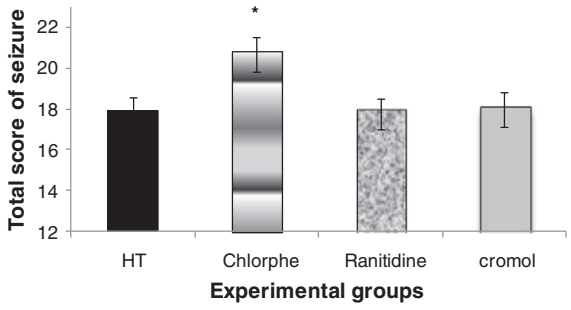

Fig. 1. The effect of different pretreatments on seizure intensity in hyperthermic infant rats. Infant rats were subjected to HT for 30 min. In the chlorpheniramine, ranitidine, and cromolyn groups, the rats were pretreated with these drugs 30 min prior to HT. * indicates $p = 0.01$ with all groups.

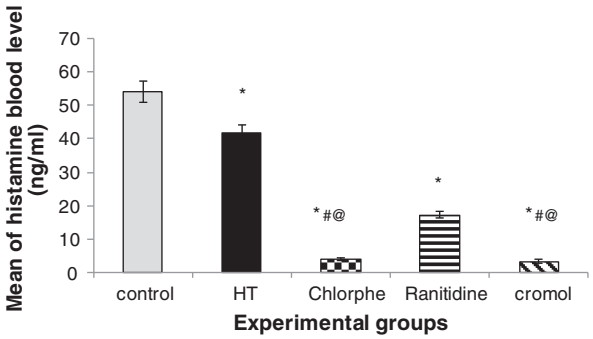

Fig. 2. The effect of hyperthermia (HT) on HBL in infant rats. Infant rats were subjected to HT for 30 min. In the chlorpheniramine, ranitidine, and cromolyn groups, the rats were pretreated with these drugs 30 min prior to HT. After HT, blood samples were obtained, and HBL was determined. * indicates $p < 0.001$ with the control group, # indicates $p < 0.001$ with the HT group, and @ indicates $p < 0.001$ with the ranitidine group.

4.3. Effect of ranitidine on HS

Both H_1 and H_2 receptor mRNAs have been detected in rat brains under normal cell culture conditions [28]. Histamine-2 antagonists are also known to cause neurological symptoms, but the incidence is lower than H_1 receptor antagonist induced problems [29]. The current results showed no significant effect on HS in the ranitidine group compared to the HT group. Also, the protective effect of L-histidine, a histamine precursor, against electrically induced clonic convulsions in mice has been abolished by H_1-receptor antagonists but not H_2-receptor antagonists [7]. The present result is in agreement with the existing literature.

4.4. Effect of HT on histamine and its effect on the existing HS

It has been reported that febrile children without seizures have a significantly higher histamine concentration than children with FS, while nonfebrile children with and without seizures have similar histamine concentrations. The increased susceptibility to seizures during fever may be related to a decrease of the CSF histamine level in children with FS [3]. It is reported that acute immobilization stress increases plasma histamine concentration about 3 times of that in freely moving rats [30]. The histamine content of the skin of Wistar rats after 15 min or more of exposure to water immersion stress is 20% lower than that of control rats, suggesting that skin mast cells dramatically respond to stress and release histamine [31]. The present results showed that HT led to a decrease in HBL both in infant and adult rats, which are not in line with these investigations. It might be concluded that HT as a stressor could lead to the release of histamine from different sources, but because of the short half-life (1–2 min) of histamine [32], HBL could be decreased during 30 min of HT, with a 5-min recovery period prior to blood sampling. In the current study, the pretreatments greatly decreased HBL in infant rats. The lowest HBL was seen in the cromolyn group, but seizure intensity in this group did not show any significant difference compared with the HT group (Fig. 1). Cromolyn cannot cross the BBB [33,34], and it is likely that it could not affect HS because of its inability to enter the brain. As Huang et al. reported, nedocromoline (a mast cell stabilizer) dose-dependently inhibited stress-induced elevation in plasma histamine so that treatment with a dose of 10-mg/kg nedocromoline reduced the basal level of histamine to 72% compared to the control level [30]. There is evidence that IP administered cromolyn attenuates seizure in mice [24]. As explained here, cromolyn may have both pro- and anticonvulsant effects simultaneously with different mechanisms. Therefore, it could not affect HS remarkably in the present study. In one study, administration of ranitidine to patients with psoriasis led to a decrease in plasma histamine and total histamine in 100-mg wet skin [35], which is consistent with the

Table 4
The comparison of the seizure parameters between pup and adult rats.

	Group				Significance
	Control pup	HT pup	Control adult	HT adult	
Baseline temperature	36.37 ± 0.13	36.38 ± 0.15	36.12 ± 0.21	36.16 ± 0.18	$p > 0.08$
Final temperature	36.43 ± 0.15	42.95 ± 0.03*	36.18 ± 0.20	42.93 ± 0.04*	$p < 0.001$, ANOVA, with controls
Immobility latency	–	8.62 ± 0.46	–	15.50 ± 0.65*	$p < 0.001$, *t* test
Circling latency	–	16.12 ± 0.25	–	28.25 ± 1.16*	$p < 0.001$, *t* test
Intensities	–	15.42 ± 0.34	–	7.08 ± 0.49*	$p < 0.001$, *t* test

The rats were exposed to HT for 30 min, and their core body temperature was recorded by a rectal probe. Control rats were placed in a hyperthermic chamber, but they were not exposed to HT.
* indicates $p < 0.001$ with control groups.

present results. There are several possibilities for the interaction of HT and histamine: HT has both inhibitory and excitatory effects in the brain; the final outcome favors the excitatory effect [2]. Interleukin-1b (IL.1β) increases during FS in the hippocampus, and the antagonist of IL.1β receptor inhibits FS [36]. The receptors for IL.1β have been found at a high density in the hippocampus and colocalize with the NMDA receptors that mediate the fast action of glutamate [37]. Hyperthermia leads to increased glutamate in CSF, and facilitates seizure initiation and propagation via NMDA receptors [23]. Histamine can decrease glutamate release, and change the balance between inhibition and excitation in favor of inhibition [38]. Hyperthermia results in hyperventilation and respiratory alkalosis which in turn increase neuronal excitability and tend to initiate seizure [39,40]. Histamine may induce a contraction in bronchial smooth muscles and subsequently traps the CO_2 in lungs. Finally, it attenuates respiratory alkalosis and the resulting neuronal excitability.

4.5. Effect of age on HT and HS

Most FS occur between 6 months and 3 years of age with the peak incidence at 18 months. Most data support the unique age specificity of the maturing brain's sensitivity to fever [41]. Although the mechanism of this increased susceptibility is unclear, animal models suggest enhanced neuronal excitability during normal brain maturation [42]. So, maturational changes in the neurotransmitter and other systems that modulate the balance of excitation and inhibition early in postnatal life influence the susceptibility of the immature brain to seizure [43]. During the maturation process, histamine changes as well [44]. Several early reports have indicated that the HBLs in children and adult humans are almost in the same range [45,46]. These reports also indicate a mild increase in the HBL of children compared to that of the adult subjects. The finding in the present work is in agreement with the result of the abovementioned study. Meanwhile, our results indicated that none of the adult rats showed HS. It also indicated that HBL was significantly lower in control adults than in control pups. Hyperthermia led to a significant decrease in HBL, both in pups and adults. Although HT could lead to hyperventilation in adult animals [47] and a decrease in HBL (the present result), there is no evidence of HS and/or FS in adult subjects. The exact mechanism of this resistance to HS and/or FS is yet unknown, but general stability and less excitability of the adult brain may be the reason. Mitchell and Cass reported that the histamine level in the blood of healthy children (9 years old) was less than that in the blood of healthy newborn infants [45]. This finding is consistent with the present results, indicating that HBL decreases with age.

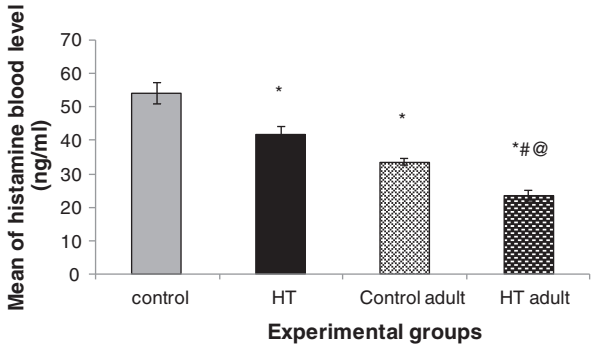

Fig. 3. The effect of HT on the HBL of the rats. The rats were subjected to HT for 30 min. Then, their blood sample was obtained, and HBL was determined. * indicates $p < 0.001$ with the control group, # indicates $p < 0.001$ with the HT adult group, and @ indicates $p = 0.003$ with the control adult group.

In general, it can be concluded that HT leads to a decrease in HBL which, in turn, increases HS susceptibility of animals. Hyperthermia-induced seizure may have several mechanisms, one of which can be the diminished histamine level due to HT. The groups pretreated with cromolyn sodium, chlorpheniramine, and ranitidine had remarkably decreased HBL compared to the HT group, while chlorpheniramine by itself potentiated the HS. Hyperthermia leads to decreased HBL without any HS in adult rats, which indicates that histamine might be a factor in inducing HS, but it is not sufficient. There are other missing factors for initiating HS, but they might not be enough (or might not even exist) in adult animals. More studies are required to clarify the details. Antihistamines are reported to be closely associated with seizure activities not only in children with epilepsy but also in healthy children [9]. According to the findings of the present study and the existing literature, clinicians should be cautious when using antihistamines on children at risk of seizure.

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